# RECENT INVESTIGATIONS UPON THE EMBRYO SAC OF ANGIOSPERMS.

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THE development of the embryo sac of the angiosperms has engaged the attention of many botanists from Hofmeister on, and the homologies of its parts have been widely discussed. All of the earlier researches seemed to indicate an extraordinary uniformity in the structures of the embryo sac, very few deviations from the type being noted, and the importance of these variations being frequently ignored.

With the improved histological methods developed in recent years there has been a renewed interest in the subject. The important paper by Treub on Casuarina[1] called attention to several striking deviations from the ordinary angiospermous type. This paper was followed by a long series of investigations by many botanists both at home and abroad, which have extended materially our knowledge both of the morphology and physiology of the embryo sac.

The uniform results of the earlier investigations may be explained, in part at least, by the selection of the more specialized forms for study. These would naturally conform, for the most part, to the structure characteristic of the embryo sac of the typical angiosperms. The more generalized and presumably more primitive forms were neglected, and the significance of such important variations as the largely increased number of antipodal cells in many grasses was usually overlooked. So great, indeed, was the uniformity assumed to be, that all efforts to explain the origin of the angiosperms from lower forms was considered well-nigh hopeless.

The studies of the last dozen years have shown that there is much more deviation from the type than was supposed to be the

[1] Sur les Casuarinées et leur place dans le système naturel, *Annales du Jardin Botanique de Buitenzorg*, tome x, pp. 143–231. Leyden, 1891.

case, and these have encouraged further researches upon the simpler types of angiosperms. Some of these have yielded interesting results, and already give some hints of the possible origin of the structures of the angiospermous embryo sac.

While it cannot be said that this very important point is likely to be explained satisfactorily in the near future, nevertheless it can be said that not unimportant advances have been made, and the purpose of this sketch is to bring together the most noteworthy results of these recent investigations, and to indicate their bearing upon the question of the origin of the angiosperms.

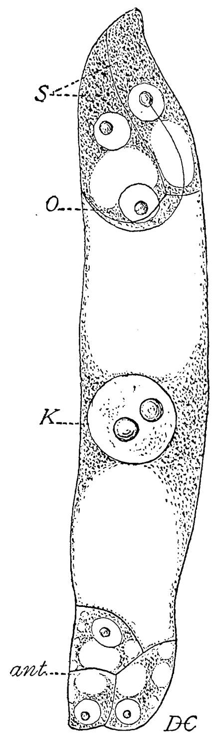

FIG. 1. — Embryo sac of *Monotropa uniflora*. At the micropylar end is the egg apparatus, consisting of the egg, *o*, and the two synergidæ, *s*; at the chalazal end, the three antipodals, *ant.*; the endosperm nucleus, *k*, still shows the two nucleoli of the polar nuclei of which it is formed.

In the great majority of investigated angiosperms the embryo sac, as is well known, exhibits extraordinary uniformity in its structure. In most instances the embryo sac arises from a subepidermal cell (Fig. 2 A), which may either develop at once into the embryo sac, or, as is more commonly the case, it divides into several cells, one of which becomes the embryo sac.

With the first division of the nucleus of the young embryo sac, the polarity which is so marked a feature becomes established. Of the two nuclei resulting from the division of the primary nucleus, one moves to the apical (micropylar) end of the sac, the other to the basal (chalazal) end. Two nuclear divisions follow, resulting in four micropylar and four chalazal nuclei. From each of these groups one nucleus, the polar nucleus, moves toward the center of the sac, where it unites with the corresponding nucleus from the opposite end. The three remaining apical nuclei, with their accompanying cytoplasm, constitute the egg apparatus; the three basal ones,

which usually become surrounded by definite cell membranes, form the three antipodal cells. The polar nuclei, either before or after fertilization, fuse to form the primary endosperm nucleus.

The most marked deviations from the typical development observed by the earlier investigators were an increase in the number of archesporial cells (Rosa *sp.*, Helleborus, etc.) and an increase in the number of antipodal cells. Of the latter variations the most marked examples were various species of grasses, first noted by Hofmeister. Further examples of both of these

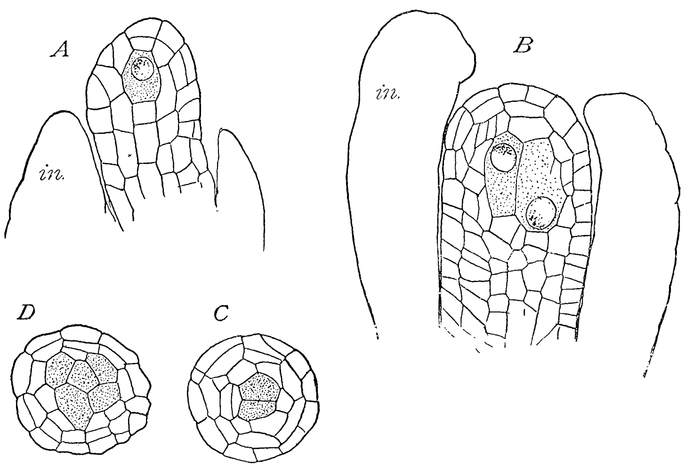


FIG. 2.—Ovules of *Arisæma triphyllum*: *A*, *B*, in longitudinal section; *C*, *D*, transverse sections of the nucellus; the archesporial cells are shaded.

deviations from the type have been recorded by later investigators, and other modifications, not hitherto observed, have been discovered.

The departures from what may be termed the typical development may be referred to several categories. Besides those already referred to, these are three in number: (1) an increase in the number of nuclei before fertilization has taken place; (2) peculiarities in the formation of the endosperm; (3) the so-called double fertilization.

The increased number of archesporial cells observed by Strasburger in *Rosa livida*, *Helleborus cupreus*, and other forms,[1] has been found to occur in a considerable number of forms.

[1] Goebel. *Outlines of Morphology*, etc.

Treub describes a large number of sporogenous cells in Casuarina, several developing into complete embryo sacs. The multicellular archesporium in this case is comparable to that of the pteridophytes and gymnosperms. This increase in the number of sporogenous cells is seen in a lesser degree in *Arisæma triphyllum* (Fig. 2). In the latter the original hypodermal archesporial cell divides longitudinally into 2–4 (occasionally more). One of these, as a rule, becomes at once the embryo sac, but sometimes there are first transverse divisions. It is possible that there may sometimes be more than one primary archesporial cell in Arisæma, *i.e.*, the whole sporogenous tissue may not certainly be referable to the division of a single hypodermal cell. This point, however, needs further investigation.

## CASUARINA.

The very peculiar genus Casuarina, according to Treub's investigations,[1] differs decidedly from the other angiosperms, not only in the large development of the sporogenous tissue of the ovule, but also in the structure of the developed embryo sac. This has no antipodal cells, and all the cells of the egg apparatus, which are variable in number, are formed from the division of a primary cell. The egg, moreover, is surrounded by a cellulose membrane before fertilization takes place, a condition unknown among other angiosperms. Before the egg cell divides (and Treub believes before it is fertilized) there is a rapid increase in the number of endosperm nuclei. A demonstration of the actual fertilization was not made, and this point must remain for the present in doubt. Should it be verified, it might well be compared with the condition existing in Peperomia.

## PEPEROMIA.

In the genus Peperomia the writer[2] found that there is normally a doubling of the nuclei of the embryo sac before fertilization; *i.e.*, instead of the eight nuclei usually found in the mature embryo sac, there are sixteen (Fig. 3). This fact

[1] *Loc. cit.*

[2] Campbell. The Embryo Sac of Peperomia, *Annals of Botany*, vol. xv (March, 1901), pp. 103–118.

was confirmed by Johnson,[1] who also discovered that the endosperm nucleus was the product of the fusion of several nuclei, instead of two, as in the typical angiosperms. In Peperomia there is no clearly defined egg apparatus, nor are there any proper antipodal cells, although a varying number of nuclei become enclosed in cell membranes and form small flattened cells applied to the wall of the embryo sac. It is to be hoped that a further study of the embryo sac of Casuarina may be made, employing carefully stained microtome sections. A comparison with the embryo sac of Peperomia would be of great interest.

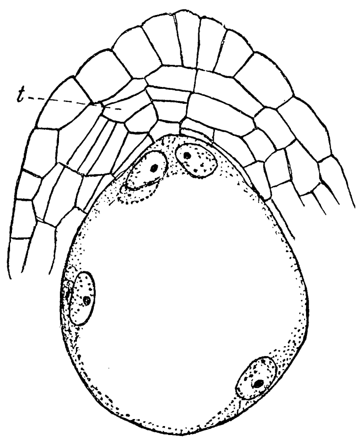


FIG. 3. — A nearly medium longitudinal section of the embryo sac of *Peperomia pellucida*: there were present sixteen free nuclei, of which six are visible in this section.

The increased number of nuclei in the embryo sac of Peperomia, while intermediate in character between that of the typical angiosperms and the lower vascular plants, might equally well be compared with that of the gymnosperms or the heterosporous pteridophytes. The nearest approach to it is found in Gnetum,[2] where the structure is not dissimilar, no true archegonium being present, but the egg cell being developed from one of the free nuclei. However, as the affinities of Gnetum are very obscure, this resemblance does not necessarily imply any connection between Peperomia and the typical gymnosperms.

An increased number of nuclei in the unfertilized embryo sac has been recorded as an occasional variant in several low monocotyledons, — *e.g.*, Naias, Zannichellia, Sparganium, — but in all these forms the normal embryo sac is of the ordinary angiospermous type.

[1] Johnson. On the Endosperm and Embryo of Peperomia pellucida, *Botanical Gazette*, vol. xxx, July, 1900.

[2] Lotsy. Contributions to the Life-History of the Genus Gnetum, *Annales du Jardin Botanique de Buitenzorg*, tome xvi (1899), pp. 46–114.

Karsten. Beiträge zur Entwickelungsgeschichte der Gattung Gnetum, *Botanische Zeitschrift*, Bd. l, 1892.

## Variation in the Antipodal Cells.

The three antipodal cells commonly found at the chalazal end of the embryo sac are frequently looked upon as merely vestigial structures, as in many cases they doubtless are. That they may be of importance physiologically has been

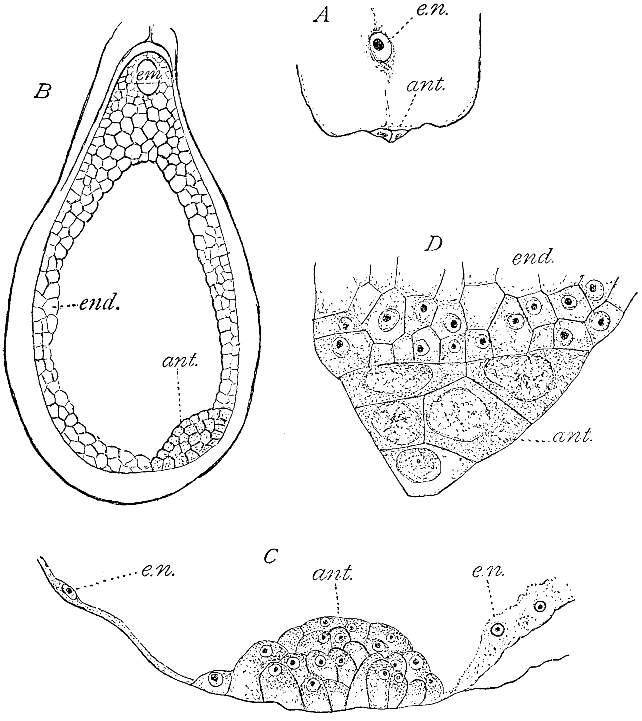


Fig. 4. — *A*, the chalazal end of the embryo sac of *Sparganium simplex*, about the time of fertilization: *e.n.*, endosperm nucleus; *ant.*, two of the three antipodal cells. *B*, section of a young seed of the same species: *em.*, embryo; *end.*, endosperm; *ant.*, the large mass of antipodal cells. *C*, antipodal cells and young endosperm from a somewhat earlier stage, more highly magnified. *D*, chalazal end of the embryo sac from a young seed of *Lysichiton kamtchatcense*, showing endosperm, *end.*, and the enlarged antipodal cells, *ant.*

repeatedly shown, and there is no question that this is oftener the case than was formerly supposed. In many forms — *e.g.*, Naias, Lilæa — the antipodal cells are large, with abundant cytoplasm, and every indication of being active cells. In other cases, such as some Ranunculaceæ,[1] they become very large,

[1] Mottier. Contributions to the Embryology of the Ranunculaceæ, *Botanical Gazette*, vol. xx, 1895.

and become multinucleate. An increase in the number of antipodal cells was observed as a regular phenomenon in various grasses, by Hofmeister, and has been noted also by later observers, — Cannon[1] found in *Avena fatua* as many as thirty-six. There are in these cases the original three antipodals which subsequently increase by division, this occurring before the egg is fecundated.

The greatest number of antipodal cells yet recorded occurs in *Sparganium simplex* (Fig. 4), where there may ultimately

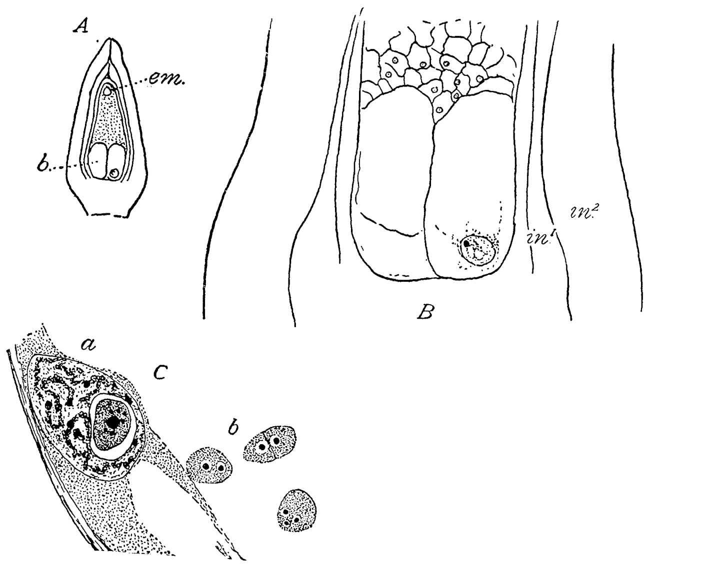


FIG. 5.—*A*, section of young seed of *Spathicarpa sagittæfolia: em.*, embryo; *b.*, enlarged basal endosperm cells, × 6. *B*, basal portion of the embryo sac of the same, more enlarged. *C*, the large basal endosperm nucleus, *a*, and the ordinary ones, *b*, from the embryo sac of *Naias flexilis*.

be 150 or more. At the time of fertilization there are but three, small and inconspicuous antipodal cells; but after fertilization these increase rapidly in size and begin to divide actively, forming a very conspicuous hemispherical mass of cells at the base of the embryo sac. This large group of antipodal cells develops while the endosperm is still rudimentary and is doubtless of great physiological importance.

[1] Cannon. A Morphological Study of the Flower and Embryo of the Wild Oat, *Proc. California Acad. of Sciences*, third series, vol. i, No. 10, 1900.

Among other similar cases may be mentioned that of the peculiar aroid, Lysichiton, which occurs upon the northern shores of the Pacific. The original antipodal cells divide in this case also, and their nuclei become enormously enlarged (Fig. 4 *D*).

From a study of the numerous instances which have been studied, it is clear that the antipodal cells are by no means merely vestigial structures, but are often of great physiological importance, replacing the endosperm to some extent, or acting as a medium for the transport of nutriment to the developing embryo.

## THE ENDOSPERM.

In the typical angiosperms the primary endosperm nucleus resulting from the fusion of the polar nuclei divides to form many free nuclei lying in the peripheral layer of cytoplasm. Walls form between these nuclei, and a layer of tissue is thus developed, lining the embryo sac. By subsequent centripetal divisions the embryo sac becomes finally completely filled with endosperm. When the embryo ultimately fills the whole embryo sac the cell divisions in the endosperm may be entirely suppressed (*e.g.*, Naias). In other cases the first division of the primary endosperm nucleus is followed by a cell wall extending across the cavity of the embryo sac, which is thus filled from the first with tissue.

A modification of the type has been noted in a number of low monocotyledons. Of the two nuclei resulting from the division of the primary endosperm nucleus, only the upper one divides further. The lower one, which may be separated by a wall from the upper part of the embryo sac,[1] sometimes becomes enormously enlarged (Fig. 5 *C*) but does not divide further. It is possible, although this has not been demonstrated, that in some cases there is no fusion of the polar nuclei.

A condition somewhat intermediate between the ordinary form and that just described was recently observed by the writer in *Spathicarpa sagittæfolia*, a South American aroid. In this case (Fig. 5) a group of a few very large cells, with

[1] Schaffner. The Life History of Sagittaria variabilis, *Botanical Gazette*, vol. xxiii, 1897.

enormous nuclei, lies at the base of the endosperm and is sharply separated from the small-celled endosperm in the upper part of the embryo sac. It is probable that the basal group of large cells is the product of the lower of the two nuclei derived from the first division of the endosperm nucleus.

In the genus Peperomia the very large endosperm nucleus is the result of the fusion of several (usually eight) nuclei. It then divides to form the rudimentary endosperm found in the ripe seed.

## Double Fertilization.

Much interest has been aroused by the discovery of the so-called double fertilization which has been demonstrated for a large number of angiosperms. This double fertilization consists in the fusion of the second generative nucleus of the pollen spore with the endosperm nucleus, which thus becomes the product of three nuclei. This has led to a theory that the endosperm nucleus is sexual in its nature, and the endosperm arising from it is an embryo. That this view can hardly be maintained is evident from the condition found in Peperomia, which presumably represents a more primitive condition than that of the typical angiosperms. In Peperomia, as already stated, the endosperm nucleus is the product of several similar nuclei, and as such a multiple fusion of sexual cells is quite unknown elsewhere, it is safe to assume that this fusion is not of the nature of a true fertilization.

That the second generative nucleus discharged into the cavity of the embryo sac should fuse with the only available nucleus, the endosperm nucleus, is not surprising. That its character is impressed upon the resulting nuclei is also to be expected. This is shown most clearly in the case of hybrid maize. It has been clearly demonstrated that one type of maize pollinated with another will produce ears in which the endosperm of the grains shows a hybrid character, due presumably to the fusion of one of the pollen nuclei with the endosperm nucleus.[1]

[1] Webber. Xenia, or the Immediate Effect of Pollen in Maize. U. S. Department of Agriculture, Division of Vegetable Physiology and Pathology, *Bulletin No. 22*, 1900.

## Summary.

Peperomia, in regard to the embryo sac, probably represents the most primitive form yet described among the angiosperms. The absence of a definite egg apparatus and antipodals, and especially the increased number of nuclei, point to this. The single-celled archesporium of Peperomia is, however, probably less primitive than the multicellular sporogenous complex found in Casuarina and some other forms.

The similarity between the structure of the embryo sac in Peperomia (and perhaps Casuarina) and Gnetum is striking; but as the affinities of the latter are very doubtful, this does not throw much light upon the relationships existing between gymnosperms and angiosperms.

The typical embryo sac may very well have been derived from one like that of Peperomia, by the suppression of one nuclear division. The marked polarity, and the specialization of the egg apparatus and antipodal cells, are probably secondary characters, and the fusion of the polar nuclei has its prototype in the multiple fusion of the nuclei in Peperomia, to form the endosperm nucleus.

Peperomia offers a basis for an explanation of the homologies of the embryo sac. The egg cell probably represents an archegonium reduced to a single cell, and possibly the synergidæ may also represent potential archegonia, although it is quite as likely that they are derived from vegetative prothallial cells.

All of the other structures, the polar nuclei (and their product, the endosperm nucleus) and the antipodal cells, represent vegetative prothallial tissue. The increase in number met with in the antipodal cells of Sparganium, for instance, merely emphasizes their power to assume the rôle of active prothallial tissue.

The fusion of the polar nuclei is in no way to be considered as a true sexual process. The regular occurrence of a multiple fusion in Peperomia is a strong argument against such an assumption. It is much more probable that it is to be interpreted as a stimulus to further growth. The fusion of the second pollen nucleus with the endosperm nucleus must be considered as more or less accidental.[1]

[1] Strasburger. Einige Bemerkungen zur Frage nach der "doppelten Befruchtung" bei den Angiospermen, *Botanische Zeitschrift*, 1899.